is substantially free of inhibition by surfactants selected from the group consisting of sodium linear alkybenzene sulfonates, sodium alkylsulfonate esters, sodium polyoxyethylene alkylsulfate esters, sodium alkylsulfonates, soaps and polyoxyethylene alkyl ethers.--

REMARKS

Objections to the Specification

The specification has been objected to for referral to SEQ ID NO:1 rather than SEQ ID NO:2 on pages 10 and 16. The specification has been amended pursuant to the suggestion of the Examiner. Withdrawal of the objections is, therefore respectfully requested.

Rejections Under 35 U.S.C. § 112, Second Paragraph

Claims 3, 8, and 10 have been rejected under 35 U.S.C. § 112, second paragraph as being indefinite. More specifically claim 3 has been rejected as being unclear in the recitation of "translocated." The term translocated has been deleted from claim 3. As such, the rejection has been obviated and withdrawal thereof is respectfully requested. Applicants further note for the record that "inversion" is, in fact, a form of amino acid

"substitution." As such, the term "inversion" was redundant in claim 3.

Claims 8 and 10 have been rejected for recitation of "the nucleic acid of SEQ ID NO:2" with the notation that SEQ ID NO:2 is an amino acid sequence. Claims 8 and 10 have been further rejected with the assertion that "hybridizes to" is indefinite without recitation of hybridization conditions. Claims 8 and 10 have been cancelled thus obviating these rejections.

Rejections Under 35 U.S.C. § 102

Claims 3, 4, 8, 10, 15 and 16 have been rejected under 35 U.S.C. § 102 as being anticipated over Tsukamoto et al. or Yuuki et al.

The Examiner specifically asserts that "one α-amylase is equivalent in activity to another α-amylase unless the specific distinguishing characteristics are claimed." Applicants traverse this rejection and withdrawal thereof is respectfully requested.

Enzymatic "activity" is characteristic of an enzyme. Specifically, the "activity" of an enzyme is a measure of the rate of substrate turnover per unit enzyme. Alfa-amylase optimally hydrolyzes the α-1,4-glucoside bond polysaccharides under alkaline

pH conditions. As such, recitation that the enzyme is "equivalent in activity" to SEQ ID NO:2 is the recitation of a distinguishing characteristic of the enzyme, as requested by the Examiner.

In addition, claim 3 has been amended to define the activity of the encoded enzyme as hydrolyzing "1,4-α-glucosidic linkages in starches, amylose, amylopectin, and degradation products thereof and in amylose forms: glucose (G1), maltose (G2), maltotriose (G3), maltotetraose (G4), maltopentaose (G5) and meltohexaose (G6) and does not hydrolyze pullulan." New claims 20 and 21 have been added which further define the isolectric point and additional biochemical properties of the encoded protein of the present invention.

The protein of SEQ ID NO:2 is the alkaline α-amylase isolated from *Bacillus* sp. KSM-AP1378, as described in the specification. As further noted in the specification the α-amylase of *Bacillus* sp. KSM-AP1378 was first characterized in WO94/26881, as disclosed in several places in the specification. See for example, page 3 and page 12. WO '881 characterizes the enzymatic activity of the α-amylase of SEQ ID NO:2 as hydrolyzing "1,4-α-glucosidic linkages in starches, amylose, amylopectin, and degradation products thereof and in amylose

forms: glucose (G1), maltose (G2), maltotriose (G3), maltotetraose (G4), maltopentaose (G5) and meltohexaose (G6) and does not hydrolyze pullulan." WO '881 further characterizes the isoelectric point, molecular weight, pH range, temperature range, ion and surfactant stability etc. of the protein. In support of this disclosure attached here to is an excerpt from EP 0 670 367 A1 which is an English equivalent of WO '881.

Applicants do not believe that it is new matter to add this characterization of the enzymatic activity of the encoded protein of claim 3 and to add new claims 20 and 21, because the activity is an inherent property of the enzyme. It has been well settled that amending a specification or claim to include an inherent property of a compound does not constitute new matter.

As indicated in the response filed on February 26, 1999, the α-amylases of Tsukamoto et al. and Yuuki et al. do not have the same enzymatic activity as the α-amylase of SEQ ID NO:2 and thus any α-amylase encompassed by the present invention. For example, the amylase of Tsukamoto et al., i.e. 707 amylase, only shares 83.5% homology with the present amylase. In addition, the 707 amylase of Tsukamoto et al. has a pH optimum of 7, a molecular

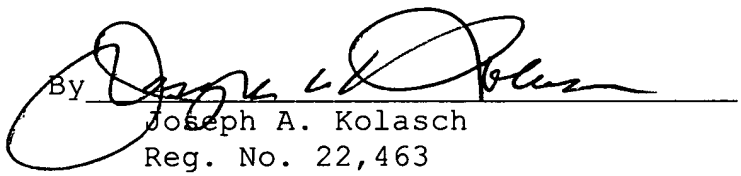
weight of 58,000 and an isoelectric point of 6.5. The amylase of Yuuki et al. has 66.5% homology with the amylase of the present invention and, as disclosed in Arch. Biochem. Biophys. 155:290-298 (1973), the amylase of Yuuki et al. has a pH optimum of 5-8 and a temperature optimum of 76°C, with a heat-resistance of 60°C. Thus, the presently claimed amylase has different enzymatic activity and different biochemical properties from the enzymes disclosed by either Tsukamoto et al. or Yuuki et al. The present invention is thus, not anticipated by either Tsukamoto et al. or Yuuki et al. and withdrawal of the rejection is respectfully requested.

As the above-presented amendments and remarks address and overcome the rejections of the Examiner, withdrawal of the rejections and reconsideration and allowance of the claims are respectfully requested. Should the Examiner have any questions regarding the present application, she is requested to contact MaryAnne Armstrong, PhD (Reg. No. 40,069) in the Washington DC area, at (703) 205-8000.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. §§1.16 or 1.17; particularly, extension of time fees.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By 
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Attachment: Excerpt from EP 670 367

[Testing method of detergency]

Washing conditions:

The washer employed: Full automatic dish washer "NP-720" (trade name, manufactured by Matsushita Electric Industries Co., Ltd.) of the type that an aqueous detergent solution is sprayed from rotary nozzles to wash dishes and the like placed on an upper surface defined by its spray flow lines.

Washing temperature: gradually raised from 5 °C to 55 °C.

Water used: water having a hardness of 3.5° DH.

Concentration of the detergent: 0.2 wt.%.

Washing time: 20 minutes washing, followed by 20 minutes rinsing.

Amount of water circulated upon washing: 2.5 ℓ.

Industrial Applicability

The liquefying alkaline α-amylase according to the present invention has liquefying activity which permits high-random degradation of substrates, such as starches and starchy polysaccharides, than the conventional alkaline α-amylases. It has an optimum pH on the alkaline side (8.0-10.0) and moreover, is extremely stable in a still wider pH range. Its optimum temperature is 45 °C-55 °C so that it retains excellent thermal stability up to 50 °C. Furthermore, its activity is hardly inhibited by other detergent components such as a surfactant. The conventionally-known alkaline α-amylases have an isoelectric point of about 3.0-8.0, while the liquefying alkaline α-amylase according to the present invention has an exceptionally high isoelectric point exceeding 8.5 (8.7-9.7, specifically around 9.2). Making use of such characteristics of this enzyme, it can readily be obtained in a purified form by gel isoelectric focusing electrophoresis, density gradient isoelectric point, ion exchange chromatography or the like. It has therefore an extremely great industrial significance.

Detergent compositions containing the liquefying alkaline α-amylase of the present invention have excellent detergency especially against the soil of smeared food.

The superior detergency of the liquefying alkaline α-amylase according to the present invention to the conventional amylases is considered to be attributable to the influence of the high isoelectric point in addition to the characteristic of the liquefying type, that is, high-random degradation. Described specifically, fibers are generally electrified negative in water. When a wash liquor has a high pH, an enzyme is also electrified negative provided that the enzyme has a low isoelectric point. As a consequence, the fibers and the enzyme become repulsive each other. The liquefying alkaline α-amylase according to the present invention, however, has a high isoelectric point so that it is not electrified negative in the wash liquor. Repulsion between the enzyme against the soil on the surface of the fiber is hence reduced, thereby probably contributing to improved detergency.

Claims

1. A liquefying alkaline α-amylase having the following enzymatic properties:
   1) Action:
      It hydrolyzes 1,4-α-glucosidic linkages in starches, amylose, amylopectin and partial degradation products thereof and from amylose, forms glucose (G1), maltose (G2), maltotriose (G3), maltotetraose (G4), maltopentaose (G5) and maltohexaose (G6). It however does not act on pullulan.
   2) Isoelectric point:
      It has an isoelectric point higher than 8.5 when measured by isoelectric focusing electrophoresis.

2. A liquefying alkaline α-amylase according to claim 1, which has an isoelectric point of 8.7 to 9.7.

3. A liquefying alkaline α-amylase according to claim 1 or 2, which has an optimum pH of from 8.0 to 10.0.

4. A liquefying alkaline α-amylase having the following enzymatic properties:
   1) Action:
      It hydrolyzes α-1,4-glucosidic linkages in starches, amylose, amylopectin and partial degradation products thereof and from amylose, forms glucose (G1), maltose (G2), maltotriose (G3), maltotetraose (G4), maltopentaose (G5) and maltohexaose (G6). It however does not act on pullulan.

2) Acting pH and optimum pH:
It acts in a pH range of from 5.0 to 11.0, with an optimum pH being 8.0 to 9.0.
3) PH stability:
It is extremely stable in a pH range of from 6.5 to 10.0 and even in a range of from pH 5.0 to 10.5. It retains at least 50% of its activity after treated at 40 °C for 30 minutes.
4) Acting temperature range and optimum temperature:
It acts in a temperature range of from 20 °C to 60 °C, with the optimum temperature being 45 °C to 55 °C.
5) Thermal stability:
It is extremely stable at temperatures of 50 °C or lower (treated for 30 minutes in a glycine-salt-sodium hydroxide buffer having pH 8.5).
6) Molecular weight:
It has a molecular weight of 50,000 ± 5,000 as measured in accordance with the sodium dodecyl sulfate polyacrylamide gel electrophoresis.
7) Isoelectric point:
It has an isoelectric point of around pH 9.2 when measured by isoelectric focusing electrophoresis.
8) Effects of metal salts:
It is extremely stable against K^+, Na^+, Ca^{2+}, Mg^{2+}, Mn^{2+}, Ba^{2+}, Fe^{2+}, Fe^{3+} and Al^{3+}.
9) Effects of surfactants:
It is substantially free from activity inhibition by surfactants such as sodium linear alkylbenzene sulfonates, sodium alkylsulfate esters, sodium polyoxyethylene alkylsulfate esters, sodium alkylsulfonates, soaps or polyoxyethylene alkyl ethers.

5. A liquefying alkaline α-amylase according to any one of the claims 1-4, which has a sequence of Asn-Gly-Thr-Met-(Met)-Gln-Tyr-Phe-Glu-Trp in its N-terminal amino acid region.

6. A process for the preparation of a liquefying alkaline α-amylase defined in any one of claims 1 to 5, which comprises culturing a liquefying-alkaline-α-amylase-producing bacterium belonging to the genus *Bacillus* and collecting from the resulting culture the liquefying alkaline α-amylase.

7. A process according to claim 6 for the preparation of the liquefying alkaline α-amylase, wherein the liquefying-alkaline-α-amylase-producing bacterium is *Bacillus sp.* KSM-AP1378 (FERM BP-3048).

8. A detergent composition comprising the liquefying alkaline α-amylase defined in any one of claims 1 to 5.

9. A detergent composition according to claim 8, wherein the liquefying alkaline α-amylase has been produced by *Bacillus sp.* KSM-AP1378 (FERM BP-3048).

10. A detergent composition according to claim 8 or 9, further comprising an anionic surfactant and/or nonionic surfactant in an amount of 0.5 to 60 wt.%.

11. A detergent composition according to any one of claims 8 to 10, wherein the liquefying alkaline α-amylase is incorporated in an amount of 1 to 10,000 U/g in terms of degrading activity against soluble starches.